

## METHOD FOR ELECTROCHEMICALLY DETECTING ANALYTE

### FIELD OF THE INVENTION

**[0001]** The present invention relates to a method for electrochemically detecting an analyte. More particularly, it relates to a method for electrochemically detecting an analyte, which is useful for detecting and quantifying analytes such as nucleic acids and proteins as well as clinically examining and diagnosing diseases using these methods.

### BACKGROUND

**[0002]** Clinical examination and diagnosis of diseases are performed by detecting genes and proteins related to the diseases which are contained in biological samples by detection methods such as a gene detection method and an immunological detection method. As a method for performing a clinical examination and diagnosis, a photochemical detection method using a photocurrent generated by exciting a photochemically active labeling substance with light to detect analytes such as nucleic acids or proteins is suggested. Here, in the clinical examination and diagnosis, it is necessary to detect a very small amount of analytes included in a specimen. Accordingly, there is a need to improve detection sensitivity of analytes.

**[0003]** For example, U.S. Patent Publication No. 2011/193187 discloses a method for specifically detecting an analyte by a photocurrent comprising: using an electrode in which an antibody, which is a trapping substance for specifically recognizing a protein as an analyte, is immobilized on the surface; and a labeled antibody in which an antibody, which is a binding substance, is labeled with an electrochemically active labeling substance. In the method described in U.S. Patent Publication No. 2011/193187, the analyte is brought into contact with the antibody on the electrode, and the analyte is trapped on the electrode by the antibody. Thereafter, the analyte trapped on the electrode is brought into contact with the labeled antibody to form a complex. Then, the analyte is detected by measuring the photocurrent based on the labeling substance in the labeled antibody.

**[0004]** However, as described in U.S. Patent Publication No. 2011/193187, when the binding substance is directly labeled with the labeling substance not via a support, there is a limit on the number of the labeling substance which can be bound to the binding substance. Thus, in the method described in U.S. Patent Publication No. 2011/193187, the number of the labeling substance per analyte cannot be increased beyond a limit, which results in difficulty in achieving high sensitivity.

**[0005]** In the method described in U.S. Patent Publication No. 2011/193187, since the complex formed on the electrode is bulky, a distance between the labeling substance included in the complex and the electrode becomes longer physically. Thus, the transportation of electrons between the labeling substance and the electrode is hardly performed. For example, an IgG antibody has a size of about 10 nm. In the method described in U.S. Patent Publication No. 2011/193187, when the IgG antibody is used as an antibody constituting a trapping substance and a labeled antibody, the labeling substance in the complex to be formed on the electrode is present in a position very distant from the electrode surface where the transportation of electrons may be efficiently occurred in detecting the analyte. Consequently, in the

method described in U.S. Patent Publication No. 2011/193187, the specific volume of the complex formed in detecting the analyte is a large restriction in terms of achieving high sensitivity.

### SUMMARY OF THE INVENTION

**[0006]** The scope of the present invention is defined solely by the appended claims, and is not affected to any degree by the statements within this summary.

**[0007]** The present invention has been achieved in view of the above circumstances. Its object is to provide a method for electrochemically detecting an analyte which can detect the analyte with high detection sensitivity.

**[0008]** The present inventors have found that the detection sensitivity can be significantly improved by binding a labeling substance which is used to detect an analyte to a binding substance via a support composed of polypeptide in the method for electrochemically detecting an analyte, and the above-described problems can be solved. Thus, the present invention has been completed.

**[0009]** Further, the present inventors have found that the detection sensitivity can be significantly improved by using a label binding substance in which a labeling substance is immobilized on an antibody via a modulator which generates an interaction with a working electrode site except a site where an electrolytic solution and a trapping substance are bound in detecting the analyte. Thus, the present invention has been completed.

**[0010]** A first aspect of the present invention is a method for electrochemically detecting an analyte comprising:

**[0011]** bringing a sample containing an analyte into contact with a working electrode on which trapping substance for trapping the analyte is immobilized to allow the analyte to be trapped on the working electrode by the trapping substance;

**[0012]** forming a complex containing the analyte trapped on the working electrode in the trapping process and a label binding substance in which a labeling substance and a binding substance for trapping the analyte are at least retained by a support composed of polypeptide; and

**[0013]** electrochemically detecting the labeling substance present on the working electrode obtained by the complex formation process.

**[0014]** A second aspect of the present invention is a method for electrochemically detecting an analyte in an electrolytic solution comprising:

**[0015]** bringing a sample containing an analyte into contact with a working electrode on which trapping substance for trapping the analyte is immobilized to allow the analyte to be trapped on the working electrode by the trapping substance;

**[0016]** forming a complex containing the analyte trapped on the working electrode in the trapping process and a label binding substance in which a labeling substance is retained via a modulator which generates an interaction with an electrolytic solution and a working electrode site except a site where the trapping substance are bound on a binding substance which binds to the analyte on the working electrode; and

**[0017]** electrochemically detecting the labeling substance present on the working electrode obtained in the complex formation process.